

pronounced than for the other cells. There is another difference in the cells of FIG. 4 from FIG. 5. The values for concentrations below 0.1 mM in FIG. 1 fluctuate between 0 and +50 enhancement of GFP with large error bars, whereas the values in FIG. 2, for the same region of concentration, all show (except for 1 point) negative GFP enhancement (i.e., in the suppression of expression region). Thus, the behavior of Cohex for the different cell types exhibit differential amounts of viral expression decrease, but they all show decreasing levels of GFP fluorescence with increasing Cohex concentrations, especially above 0.1 mM.

**[0055]** In order to check whether the decreasing GFP levels were simply due to decreasing numbers of viable cells, in vitro cytotoxicity studies were performed for the same cell lines. That is, the same concentration ranges as used above were used in a CellTiter-Glo Luminescent Cell Viability Assay by Promega. This assay is based on quantitation of the ATP present in cells, which signals the presence of metabolically active cells, that is, a decrease in luminescence correlates with a decrease in the number of viable cells. The cells were plated out on 96-well plates, as above, and incubated at 37° C. for 24 hours before adding compound. The treated cells were then allowed to grow for an additional 48 hours before reading on the BMG Lumistar set on the ATP protocol.

**[0056]** In addition to the luminescence assay, a flow cytometry assay was performed using propidium iodide as a “dead” stain for A549 cells. The flow cytometry assay protocol for A549 cell line is similar to protocols known in the art, and is as follows. The cells were grown until confluent and reseeded at 100,000 cells/well in 1 ml in 24-well plates. The monolayers were allowed to form overnight at 37° C. under 5% CO<sub>2</sub>. The Cohex dilution series was added to appropriate wells and the plate incubated for 48 hours at 37° C. under 5% CO<sub>2</sub>. The cells were then washed, pelleted, resuspended in buffer, and transferred to BD falcon tubes for flow analysis. A BD FACSort cytometer and BD CellQuest software was used to quantify cell viability. Prior to flow analysis, 10 µL of propidium iodide (PI) at 0.05 mg/ml was added to each tube to stain dead cells. Analysis was performed on 1×10<sup>4</sup> events/well.

**[0057]** FIG. 6 shows the result of the cytotoxicity assay for A549 and HepG2 cells plotted on semi-log scale. There appears to be no toxic effect until about 0.1 mM, after which there is a decreasing % of viable cells. To better show the region from 2.5 µM to 0.1 mM, FIG. 7 provides linear-scale plots to emphasize the concentration region that does affect cytotoxicity.

**[0058]** Both 293T and VerE6 cells lines show much less cytotoxic susceptibility to Cohex, leveling off between 70 to 80% viability, even at 5 mM Cohex. There is a variety of reactions to Cohex by different cell lines, but none of the cells were 100% killed, whereas suppression of GFP expression tends to bottom out close to -100% (except for VeroE6).

**[0059]** It is further notable that, in addition to variability between cell lines, different markers can also differ in their assessment of viability. As an example, the results of a flow cytometry measurement using propidium iodide (PI) as a marker for dead cells shown in FIG. 9. it can be seen that PI appears to measure a cell property (cell permeability) that is much less affected by Cohex than the luminescence study (ATP levels).

**[0060]** The IC<sub>50</sub> for Cohex for the different cell lines can be estimated from FIGS. 1 and 2. By using a log concentration scale, the data can be fitted to the classic sigmoidal shape

using a non-linear least-squares fitting program, seen in FIG. 10. The IC<sub>50</sub> for the fit was found to be 0.38 mM Cohex.

**[0061]** The results with various cell types are shown in Table 2.

TABLE 2

Summary of Cohex IC <sub>50</sub> for Various Cell Types				
	A549	HepG2	VeroE6	293T
IC <sub>50</sub> (mM)	0.48	0.24	1.66	1.28

#### Cohex Animal Study Against Ebola

**[0062]** An efficacy study was conducted in mice to test whether Cohex would have a therapeutic affect against Ebola virus exposure. Initially, to determine whether the mice would tolerate the Cohex, they received intraperitoneal (IP) injections of Cohex once a day for 10 days at levels of 0.5, 1, 2, 4, and 8 mg/kg in this study. The mice tolerated the compound very well, with no adverse reactions reported.

**[0063]** To examine the efficacy of Cohex, mice were treated by IP injection with either phosphate buffered saline (PBS) or Cohex in PBS one hour before virus exposure, and further treated once a day for 9 more days. In comparing the results of the mice treated with PBS versus those treated with 8 mg/kg of Cohex, it was found to be statistically very likely (p=0.01 in a chi-squared test) that the 8 mg/kg treatment improved survival rates over the PBS treatment in mice infected with Ebola virus.

**[0064]** The general advantages of a broad-spectrum drug, such as Cohex, are its low-cost, stability, and, of course, ability to attack multiple microorganisms. When there is no treatment available, as in the case of Ebola virus, Cohex could be the only source of treatment. For viruses, such as HIV, where drugs with very high TI already exist, Cohex can be used in a combination drug therapy regime. There are several advantages to doing this: (1) as a broad-spectrum compound, Cohex can fight against opportunistic infections by other microorganisms; (2) Cohex may have a synergistic effect on existing anti-HIV drugs; (3) Cohex can significantly decrease the cost of anti-HIV treatment; (4) Cohex can slow the development of viral drug-resistance by presenting a very different mechanism that must be overcome.

**[0065]** All numbers expressing quantities of ingredients, constituents, reaction conditions, and so forth used in the specification are to be understood as being modified in all instances by the term “about.” Notwithstanding that the numerical ranges and parameters set forth, the broad scope of the subject matter presented herein are approximations, the numerical values set forth are indicated as precisely as possible. Any numerical value, however, may inherently contain certain errors resulting, for example, from their respective measurement techniques, as evidenced by standard deviations associated therewith.

**[0066]** Although the present invention has been described in connection with preferred embodiments thereof, it will be appreciated by those skilled in the art that additions, deletions, modifications, and substitutions not specifically described may be made without departing from the spirit and scope of the invention. Terminology used herein should not be construed in accordance with 35 U.S.C. §112, ¶6 unless the term “means” is expressly used in association therewith.